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Interactive comment on "Evidence for microbial mediated nitrate cycling within floodplain sediments during groundwater fluctuations" *by* Nicholas J. Bouskill et al.

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Validation and statistical assessment. However, the article in its present form lacks validation through microbial isotope experiments, which would truly provide "evidence" for microbial mediated nitrate cycling.

We have added additional statistical analysis to the current work through the use of a Taylor diagram (Fig. S6). With respect to validation, the model has been tested against several different scenarios related to dilution + denitrification (added into the supplemental, Fig. S7), denitrification alone (Fig. 4 of the main text), increasing OM concentrations (Fig. S4), and varying ratios of electron donors (Fig. S5). The model captures

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the changing nitrate concentrations attributable to abiotic and biotic processes, but can also be used to solely capture the nitrate turnover attributable to denitrification (parsing this out from that attributable to dilution). Increasing the organic matter concentration shows a clear correspondence between denitrification and the decline in nitrate, however, the isotopic data suggests that denitrification is responsible for much lower nitrate reduction loss than this.

The details of that season (e.g., rather it was a normal rainfall year) were not presented.

Further information on precipitation has been added to the materials and methods. The year in which this study was conducted, 2014, had a high snowpack, which drove the water table higher than previous years. Normally the water table does not immerse 2 m bsd, however, regularly saturates the 2.5 and 3 m bsd. This information has been added to the materials and methods section.

Likely, a map of the monitoring locations would be helpful within the floodplain.

A map of the site showing the location of the study well has been added to the supplemental (Figure S1).

Further information is needed regarding the soil and water chemistry (e.g. pH), which will impact microbial community population and productivity.

Further information on pH, electrical conductivity and dissolved oxygen has been added to the results section.

The high nitrate concentrations were surprising - Is this area in an agricultural land-

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scape?

This area is not impacted by agriculture, however, has a number of heterogeneously distributed naturally reduced zones (NRZs). This aspect of the site is discussed on Pg. 14, Ln 11. The presence of these NRZs (which are essentially buried horizons formed by river overbanking), generally explains the high nitrate concentrations. Organic matter concentrations are very high in these regions, Janot et al., ES&T, 2016, recorded organic matter in these regions with OC concentrations as high as 1.7 %. We can therefore use a back-of-the-envelope calculation to estimate nitrogen availability from the OM in these regions. Considering a measured C:N ratio for the relevant depths of 7 (Conrad et al., unpublished) and a bulk density of $\sim$ 2 g cm-3, OM in these naturally reduced regions could yield 0.004 g-N cm-3, or 290 mM of nitrogen. Using a conservative mineralization rate of 2 % per year would therefore yield $\sim$ 6 mM of nitrogen.

“Evidence for microbial mediated nitrate cycling. . .” seems misleading, assuming no microbial isotopic data was collected in the soils.

The measurements reported by the current manuscript are taken from porewater samples collected under both saturated and unsaturated conditions over the course of the year. The wells drilled into the floodplain site incorporate a suction lysimeter at each depth sampled (as outlined on Pg. 4 Ln. 1), allowing for sampling across the year.

Additionally, do the authors have isotopic data for the confined aquifer to confirm that the mixing water is truly nitrate free?

Nitrate accumulates and dissipates only in the depths currently under investigation (i.e., 2 - 3 m below surface depth), with little evidence from this study or from previous studies that nitrate accumulates at shallower or deeper depths. Measurements of nitrate in the

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vadose zone were below detection (figure 2), it is also unlikely, given infiltration rates at this site ($\sim$ 3 cm yr-1, Pg. 14, Ln 2), that nitrate from shallower soils are transported to = 2 m and below. . This is further supported by recent work at the site adding $\sim$ 2500 gallons of deuterium-enriched snow (δD $\sim$ 2200 per mil), for the purpose of examine water infiltration into the vadose zone around the well used in the current study. Snowmelt last 6 days and δD rapidly infiltrated to $\sim$ 1 – 1.5 m, with very little deuterium signal seen below 1.5 m. Therefore, the transportation of nitrate from the vadose zone to the capillary fringe was not considered to be of importance in the current study. Similarly, nitrate below the 3 m line has been shown to be very low. Fig. 2 shows nitrate data for 3.14 m below surface depth, the lower bound of the current data set, with nitrate concentration ranging from 60 to 700 micromoles. Below this, into the background aquifer, nitrate ranges from undetectable up to 80 micromoles, as reported in previous studies (Zachara et al, J. Cont. Hydrol. 2013; Yabusaki et al., ES&T, 2017). This is alluded to in the main text (Pg. 3, Ln 14), however, we have rewritten this statement to make it clearer.

Finally, and further emphasizing the nitrate-deficient conditions in the groundwater, a recent nitrate injection experiment injected $\sim$ 2 mM of nitrate into the groundwater intending to stimulate chemolithoautotrophic metabolisms (Jewell et al., ISME, 2016; Frontiers in Microbiology, 2016). Prior to the injection, nitrate concentrations ranged from undetectable to $\sim$ 70 μM. Post-injection, the nitrate was entirely consumed within the first 1 m downgradient.

In summary, the reason that no isotope measurements were made in the vadose zone or background aquifer was that nitrate was often below detection limits of the technique. This would also minimize the likelihood of nitrate from outside the depths studied contributing significantly to the observations reported here.

Based on purely visual observations, the model predications do not appear to fit well

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with the observed data

The model (as shown in Fig. 4) is representing only biological nitrate loss (i.e., attributable to denitrification or anammox). One of the points that we wish to make with this manuscript is that a model can be forced to replicate data (Figure S3 is an example of this), however, the right results can emerge for the wrong reasons. This emphasizes the importance of using the correct data to benchmark the model. In the current study, simulations presented in figure 4 reflect the qualitative conclusions of the isotopic data (i.e., denitrification is not, and cannot, be responsible for all nitrate loss). These simulations show relatively poor correlation to the observations (as shown through statistical comparisons), yet are valuable for making the point that the correct benchmarks are important.

We have added an additional paragraph to the conclusions reflecting this point. However, additional simulations are included in the supplemental material showing the comparison between observations and simulations when dilution is incorporated at the same time. As expected, this set of simulations shows excellent correlation to the observations. However, the earlier point stands, that setting up the model to simulate in well N-species, and OM concentrations can replicate the extent of nitrate reduction as informed by isotopic data. This is a significant advantage of the current model.

The second sentence in section 4.1 needs a reference. A reference has been provided.

Additionally, several of the graphs are difficult to read (e.g. Fig 4, S3, S4). We acknowledge that these figures are complicated, and attempts have been made to improve the contrast within the figures.

Please also note the supplement to this comment:

https://www.biogeosciences-discuss.net/bg-2017-212/bg-2017-212-AC2-supplement.pdf

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